JC13 Rec'd PCT/PTO 06 APR 2001

FORM PTO-1390 (REV. 11-2000) U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	
2921-0130P	
U.S. APPLICATION NO. (If known, see 37 CFR 1.5)	
09/807007 NEW	
INTERNATIONAL APPLICATION NO.	**INTERNATIONAL FILING DATE**
PCT/SE99/01784	October 6, 1999
PRIORITY DATE CLAIMED	
October 6, 1998	

TITLE OF INVENTION

A NOVEL COMPONENT IN THE HEDGEHOG SIGNALLING PATHWAY

APPLICANT(S) FOR DO/EO/US

ZAPHIROPOULOS, Peter G.; UNDEN, Anne Birgitte; TOFTGARD, Rume; RAHNAMA, Fahimeh; *

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39 (1).
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau). WO 00/20037
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☐ is transmitted herewith.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4)
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 20. below concern document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98./International Search Report (PCT/ISA/210)
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
14. ☐ A SECOND or SUBSEQUENT preliminary amendment.
15. ☐ A substitute specification.
16. ☐ A change of power of attorney and/or address letter.
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821-1.825.
18. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. ☒ Other items or information:
    1.) Sequence listing (9 pages)
    2.) 13 Pages of Drawings
    3.) PCT Substitute Claims Letter w/ International Preliminary Examination Report (PCT/IPEA/409) and Claims

*HOLLINGSWORTH, Robert E.

U.S. APPLICATION NO (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
09/807007 NEW	PCT/SE99/01784	2921-0130P

21. ☒ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5):		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO. **$1,000.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$860.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO. **$710.00**		
International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$690.00**		
International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4). **$100.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 1000.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$ 130.00	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total Claims	24 - 20 =	4	X **$18.00**	$ 72.00	
Independent Claims	1 - 3 =	0	X **$80.00**	$ 0	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)	yes		+ **$270.00**	$ 270.00	
			TOTAL OF ABOVE CALCULATIONS =	$ 1472.00	
☐ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.				$ 0	
			SUBTOTAL =	$ 1472.00	
Processing fee of **$130.00** for furnishing the English translation later than ☐20 ☐30 months from the earliest claimed priority date (37 CFR 1.492(f)).			+	$ 0	
			TOTAL NATIONAL FEE =	$ 1472.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property			+	$ 0	
			TOTAL FEES ENCLOSED =	$ 1472.00	
				Amount to be: refunded	$
				charged	$

a. ☒ A check in the amount of $ **1472.00** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account. No. _____ in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-2448.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

Send all correspondence to:
Birch, Stewart, Kolasch & Birch, LLP or Customer No. 2292
P.O. Box 747
Falls Church, VA 22040-0747
(703)205-8000

Date: April 6, 2001

By mf Neill #36,627
Gerald M. Murphy, Jr., #28,977

/GMM/ja

IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicant:	ZAPHIROPOULOS, Peter et al.	Conf.:	
Int'l. Appl. No.:	PCT/SE99/01784		
Appl. No.:	New	Group:	
Filed:	April 6, 2001	Examiner:	
For:	A NOVEL COMPONENT IN THE HEDGEHOG SIGNALLING PATHWAY		

PRELIMINARY AMENDMENT

BOX PATENT APPLICATION
Assistant Commissioner for Patents
Washington, DC 20231

April 6, 2001

Sir:

The following Preliminary Amendments and Remarks are respectfully submitted in connection with the above-identified application.

AMENDMENTS

IN THE SPECIFICATION:

Please amend the specification as follows:

Before line 1, insert --This application is the national phase under 35 U.S.C. § 371 of PCT International Application No. PCT/SE99/01784 which has an International filing date of October 6, 1999, which designated the United States of America.--

IN THE CLAIMS:

Please amend the claims as follows:

5. (Amended) A protein according to claim 1 or a nucleic acid according to claim 2 for use as a medicament.

6. (Amended) Use of a protein according to claim 1 or a nucleic acid according to claim 2 in the manufacture of a medicament for the treatment of a condition involving tumors, such as BSS.

7. (Amended) A method of in vitro or in vivo diagnosis, wherein a protein according to claim 1 or a nucleic acid according to claim 2 is used.

11. (Amended) A vector comprising a nucleic acid according to claim 2.

15. (Amended) A kit for the detection of human PTCH2 gene or polypeptide comprising in a container a molecule selected from the group consisting of a nucleic acid according to claim 2, a protein according to claim 1 or an antibody according to claim 13.

16. (Amended) Use of a nucleic acid according to claim 2 in gene therapy.

REMARKS

The specification has been amended to provide a cross-reference to the previously filed International Application. The claims have also been amended to delete multiple dependencies and to place the application into better form for examination. Entry of the present amendment and favorable action on the above-identified application are earnestly solicited.

Attached hereto is a marked-up copy of the changes made to the application by this Amendment.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By _____
Gerald M. Murphy, Jr., #28,977

P.O. Box 747
Falls Church, VA 22040-0747
(703) 205-8000

GMM/cqc
2921-0130P

Attachment: Version With Markings Showing Changes Made

(Rev. 01/22/01)

VERSION WITH MARKINGS SHOWING CHANGES MADE

The specification has been amended to provide cross-referencing to the International Application.

The claims have been amended as follows:

5. (Amended) A protein according to claim 1 or a nucleic acid according to [any one of claims 2-4]claim 2 for use as a medicament.

6. (Amended) Use of a protein according to claim 1 or a nucleic acid according to [any one of claims 2-4]claim 2 in the manufacture of a medicament for the treatment of a condition involving tumors, such as BSS.

7. (Amended) A method of in vitro or in vivo diagnosis, wherein a protein according to claim 1 or a nucleic acid according to [any one of claims 2-4]claim 2 is used.

11. (Amended) A vector comprising a nucleic acid according to [any one of claims 2-4]claim 2.

15. (Amended) A kit for the detection of human PTCH2 gene or polypeptide comprising in a container a molecule selected from the group consisting of a nucleic acid according to [any one of claims 2-4]claim 2, a protein according to claim 1 or an antibody according to claim 13.

16. (Amended) Use of a nucleic acid according to [any one of claims 2-4]claim 2 in gene therapy.

BOX SEQUENCE
PATENT
2921-0130P

IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicant:	ZAPHIROPOULOS, P. et al.	Conf.:	8337
Appl. No.:	09/807,007	Group:	Unassigned
Filed:	April 6, 2001	Examiner:	Unassigned
For:	A NOVEL COMPONENT IN THE HEDGEHOG SIGNALLING PATHWAY		

AMENDMENT

Assistant Commissioner for Patents
Washington, DC 20231

July 9, 2001
(Monday)

Sir:

In reply to the U.S. Patent Office Notice to Comply with Requirements for Patent Applications Containing Nucleotide Sequence and/or Amino Acid Disclosures dated May 8, 2001, the following amendments and remarks are respectfully submitted in connection with the above-identified application.

IN THE SPECIFICATION:

Please replace the paragraph beginning on page 5, line 22 with the following amended paragraph:

--Figure 2A discloses an amino acid sequence comparison of the human PTCH2 (residues 1-633 of SEQ ID NO:1)(upper lines) and PTCH1(residues 1-699 of SEQ ID NO:6) (lower lines) sequences.--

Please replace the paragraph beginning on page 5, line 24 with the following amended paragraph:

--Figure 2B is a representation of the alternative splicing events (SEQ ID NOS:7, 8, 9, 10, 11, 12, 13, 14, 15 and 16) that result in different C-termini.--

Please replace the paragraph beginning on page 18, line 8 with the following amended paragraph:

--Detailed description of the drawings

Figure 1 shows the genomic sequence of SEQ ID NO:5, wherein exons and introns are designated in the genomic sequence of the present human patched 2 gene. However, exons 12a and 12b discussed above are not specifically shown in Figure 1, but is instead disclosed as the separate sequences SEQ ID NO:3 and SEQ ID NO:4, respectively. Figure 2A discloses an amino acid sequence comparison of the human PTCH2(residues 1-633 of SEQ ID NO:1) (upper lines) and PTCH1(residues 1-699 of SEQ ID NO:6) (lower lines) sequences. Vertical lines indicate identical amino acids, while dots similar amino acids. The PTCH2 sequence presented is composed of the original cDNA clones and of the products of the 5' RACE analysis.--

Please replace the paragraph beginning on page 18, line 19, with the following amended paragraph:

--Figure 2B is a representation of the alternative splicing events (SEQ ID NOS:7, 8, 9, 10, 11, 12, 13, 14, 15 and 16) that result in different C-termini. In the parotid gland and the colon, the penultimate and the last exon are canonically joined together. In fetal brain however the penultimate exon with part of the 3' intron functions as the terminal exon. The intronic sequence is shown by small letters with the flanking exonic by capital letters. Above the nucleotide sequence, the deduced amino acid sequence is shown, and below is the corresponding sequence of the mouse Ptch2. The conserved intronic dinucleotides are shown by bold letters and the termination signals are indicated by asterisks. Note the absence of conservation of the position of the termination codons between the mouse and human PTCH2 sequences. The putative polyadenylation signals are also shown in this diagram. The genomic organization was obtained by analyzing BAC clones encompassing the PTCH2 gene.--

Please replace the Sequence Listing filed April 6, 2001 located immediately after the claims with Substitute Sequence Listing enclosed herewith.

REMARKS

Enclosed herewith in full compliance to 37 C.F.R. §§1.821-1.825 is a substitute Sequence Listing to be inserted into the specification as indicated above. The substitute Sequence Listing in no way introduces new matter into the specification.

Also submitted herewith in full compliance to 37 C.F.R. §§1.821-1.825 is a disk copy of the substitute Sequence Listing. The disk copy of the substitute Sequence Listing, file "2921-0130P.ST25", is identical to the paper copy, except that it lacks formatting.

The substitute Sequence Listing includes the sequences disclosed in the figures as filed that were not made part of the original Sequence Listing. The amendments to the Specification are being made to reference the sequences by their SEQ ID NOS. These amendments are editorial in nature and do not constitute new matter.

Entry of the above amendments is earnestly solicited. An early and favorable first action on the merits is earnestly solicited.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By _____ #32,868
for Gerald M. Murphy, Jr.

P.O. Box 747
Falls Church, VA 22040-0747
(703) 205-8000

GMM/KW
2921-0130P

Attachments: Paper and disk copy and of Sequence Listing
Copy of Notice to Comply
Version with Markings to Show Changes Made

VERSION WITH MARKINGS TO SHOW CHANGES MADE

The paragraph beginning on page 5, line 22 has been amended as follows:

--Figure 2A discloses an amino acid sequence comparison of the human PTCH2 (residues 1-633 0f SEQ ID NO:1)(upper lines) and PTCH1(residues 1-699 of SEQ ID NO:6) –(lower lines) sequences. --

The paragraph beginning on page 5, line 24 has been amended as folllows:

--Figure 2B is a representation of the alternative splicing events (SEQ ID NOS:7, 8, 9, 10, and 11) that result in different C-termini.--

The paragraph beginning on page 18, line 8 has been amended as follows:

--Detailed description of the drawings

Figure 1 shows the genomic sequence of SEQ ID NO:5, wherein exons and introns are designated in the genomic sequence of the present human patched 2 gene. However, exons 12a and 12b discussed above are not specifically shown in Figure 1, but is instead disclosed as the separate sequences SEQ ID NO:3 and SEQ ID NO:4, respectively. Figure 2A discloses an amino acid sequence comparison of the human PTCH2(residues 1-633 0f SEQ ID NO:1) (upper lines) and PTCH1(residues 1-699 of SEQ ID NO:6) (lower lines) sequences. Vertical lines indicate identical amino acids, while dots similar amino acids. The PTCH2 sequence presented is composed of the original cDNA clones and of the products of the 5' RACE analysis.--

The paragraph beginning on page 18, line 19, has been amended as follows:

--Figure 2B is a representation of the alternative splicing events (SEQ ID NOS:7, 8, 9, 10, 11, 12, 13, 14, 15 and 16) that result in different C-termini. In the parotid gland and the colon, the penultimate and the last exon are canonically joined together. In fetal brain however the penultimate exon with part of the 3' intron functions as the terminal exon. The intronic sequence is shown by small letters with the flanking exonic by capital letters. Above the nucleotide sequence, the deduced amino acid sequence is shown, and below is the corresponding sequence of the mouse Ptch2. The conserved intronic dinucleotides are shown by bold letters and the termination signals

are indicated by asterisks. Note the absence of conservation of the position of the termination codons between the mouse and human PTCH2 sequences. The putative polyadenylation signals are also shown in this diagram. The genomic organization was obtained by analyzing BAC clones encompassing the PTCH2 gene.--

A NOVEL COMPONENT IN THE HEDGEHOG SIGNALLING PATHWAY

Technical field

The present invention relates to novel molecules, such as proteins, polypeptides and nucleotides, involved in the hedgehog signalling pathway with putative involvement in embryonic development and carcinogenesis. The invention also relates to various novel advantageous uses of the molecules according to the invention, e.g. in diagnosis and therapy.

Background

In the study of the development of cells, fruit flies have extensively been used as a model, as they are less complex than mammalian cells.

Pattern formation takes place through a series of logical steps, reiterated many times during the development of an organism. Viewed from a broader evolutionary perspective, across species, the same sort of reiterative pattern formations are seen. The central dogma of pattern formation has been described (Lawrence and Struhl, 1996). Three interlocking and overlapping steps are defined. Firstly, positional information in the form of morphogen gradients allocate cells into non-overlapping sets, each set founding a compartment. Secondly, each of these compartments acquire a genetic address, as a result of the function of active "selector" genes, that specify cell fate within a compartment and also instruct cells and their descendents how to communicate with cells in neighboring compartments. The third step involves interactions between cells in adjacent compartments, initiating new morphogen gradients, which directly organize the pattern.

Taking these steps in greater detail, one finds the first step in patterning to be the definition of sets of cells in each primordium. Cells are allocated according to their positions with respect to both dorsoventral and anterior/posterior axes by morphogen gradients. Allocation of cells in the dorsoventral axis constitutes the germ layers, such as mesoderm or neurectoderm.

In segmentation, the second step (the specification of cell fate in each compartment) is carried out by the gene *engrailed* and elements of the bithorax complex. *Engrailed* defines anterior and posterior compartments both in segmentation and in limb specification.

The third step in pattern formation, secretion of morphogens, functions to differentiate patterns within compartments (and thereby establish segment polarity). Initially, all cells within a compartment are equipotent, but they become diversified to form pattern. Pattern formation depends on gradients of morphogens, gradients initiated along compartment boundaries. Such gradients are established by a short-range signal induced in all the cells of the compartment in which the above mentioned selector gene *engrailed* is active. For segment polarity, this signal is Hedgehog. In the adjacent compartment the selector gene is inactive, ensuring that the cells are sensitive to the signal. The Hedgehog signal range is probably only a few rows of cells wide; responding cells become a linear source of a long-range morphogen, that diffuses outward in all directions. There are three known Hedgehogs, Sonic (SHH), Indian (IHH) and Desert (DHH). The proteins they encode can substitute each for each other, but in wildtype animals, their distinct distributions result in unique activities. SHH controls the polarity of limb growth, directs the development of neurons in the ventral neural tube and patterns somities. IHH controls endochondral bone development and DHH is necessary for spermiogenesis. Vertebrate hedgehog genes are expressed in many other tissues, including the peripheral nervous system, brain, lung, liver, kidney, tooth primordia, genitalia and hindgut and foregut endoderm.

Thus, segment polarity genes have been identified in flies as mutations, which change the pattern of structures of the body segments. Mutations in these genes cause animals to develop the changed patterns on the surfaces of body segments, the changes affecting the pattern along the head to tail axis. For example, mutations in the gene *patched* cause each body segment to develop without the normal structures in the center of each segment. Instead there is a mirror image of the pattern normally found in the anterior segment. Thus, cells in the center of the segment make the

wrong structures, and point them in the wrong direction with reference to the over all head-to-tail polarity of the animal.

About sixteen genes in the class are known. The encoded proteins include kinases, transcription factors, a cell junction protein, two secreted proteins called wingless (WG) and the above mentioned Hedgehog (HH), a single transmembrane protein called patched (PTC) and some novel proteins not related to any known protein. All of these proteins are beleived to work together in signaling pathways that inform cells about their neighbors in order to set cell fates and polarities.

PTC has been proposed as a receptor for HH protein based on genetic experiments in flies. A model for the relationship is that PTC acts through a largely unknown pathway to inactivate both its own transcription and the transcription of the *wingless* segment polarity gene. This model proposes that HH protein, secreted from adjacent cells, binds to the PTC receptor, inactivates it and thereby prevents PTC from turning off its own transcription or that of *wingless*. A number of experiments have shown coordinate events between PTC and HH.

Human *patched* gene (PTCH) was recently identified as the gene responsible for the nevoid basal cell carcinoma syndrome (NBCCS), also known as the Gorlin Syndrome, which is an autosomal dominant disorder that predisposes to both cancer and developmental defects (Gorlin (1995) *Dermatologic Clinics* 13:113-125) characterized by multiple basal cell carcinomas (BCCs), medulloblastomas and ovarian fibromas as well as numerous developmental anomalities (Hahn, H., Wicking, C., Zaphiropoulos, P.G., Gailani, M.R., Shanley, S., Chidambaram, A., Vorechovsky, I., Holmberg, E., Undén, A.B., Gillies, S., Negus, K., Smyth, I., Pressman, C., Leffell, D.J., Gerrard, B., Goldstein, A.M., Dean, M., Toftgård, R., Chenevix-Trench, G., Wainright, B. and Bale, A.E. (1996): "Mutations of the human homolog of Drosophila patched in the nevoid basal cell carcinoma syndrome", Cell 85, 841-851; and Johnson, R.L., Rothman, A.L., Xie, J., Goodrich, L.V., Bare, J.W., Bonifas, J.M., Quinn, A.G., Myers, R.M., Cox, D.R., Epstein, E.H. Jr and Scott, M.P.

(1996): "Human homolog of patched, a candidate gene for the basal cell nevus syndrome", Science 272, 1668-1671). PTCH codes for a membrane receptor of the autolytically cleaved (protein spliced), amino terminal domain of *sonic hedgehog* (SHH) (Mariago, V., Davey, R.A., Zuo, Y., Cunningham, J.M. and Tabin, C.J. (1996): "Biochemical evidence that patched is the Hedgehog receptor", Nature 384, 176-179; and Stone, D.M., Hynes, M., Armanini, M., Swanson, T.A., Gu, Q., Johnson, R.L., Scott, M.P., Pennica, D., Goddard, A., Phillips, H., Noll, M., Hooper, J.E., de Sauvage, F. and Rosenthal, A. (1996): "The tumor-suppressor gene patched encodes a candidate receptor for Sonic hedgehog", Nature 384, 129-134). In the non-signalling state, PTCH is thought to inhibit the consecutive signalling of another membrane protein, *smoothened* (SMO), however binding of SHH to PTCH releives this inhibition (Goodrich, L.V., Milenkovic, L., Higgins, K.M. and Scott, M.P. (1997): "Altered neural cell fates and medullablastom in mouse patched mutants", Science 277, 1109-1113). This cascade of signalling events, best characterized in Drosophila, also involves a number of intracellular components including *fused* (a serine threonine kinase), *suppressor of fused, costal 2*, and *cubitus interruptus* (Ruiz i Altaba, A.,: "Catching a Gli-mpse of Hedgehog" (1997) Cell 90, 193-196). The latter is a transcription factor that positively regulates the expression of target genes which also include PTCH itself.

Mutations in the PTCH gene have been identified in both sporadic and familial BCCs (Gailani, M.R., Ståhle-Bäckdahl, M., Leffell, D.J., Glynn, M., Zaphiropoulos, P.G., Pressman, C., Undén, A.B., Dean, M., Brash, D. E., Bale, A.E. and Toftgård, R. (1996): "The role of human homologue of Drosophila patched in sporadic basal cell carcinomas" Nature Genet. 14, 78-81). The lack of the normal PTCH protein in these cells allows the constitutive signalling of SMO to occur, resulting in the accumulation of mutant PTCH mRNAs (Undén, B. A., Zaphiropolous, P.G., Bruce, K., Toftgård, R., and Ståhle-Bäckdahl, M. (1997): "Human patched (PTCH) mRNA is overexpressed consistently in tumor cells of both familial and sporadic basal cell carcinoma", Cancer Res. 57, 2336-2340).

WO 96/11260 discloses the isolation of *patched* genes and the use of the PTC protein to identify ligands, other than the established ligand Hedgehog, that bind thereto.

However, there is still a need of a further understanding of the SHH/PTCH cell signalling, which may be provided by disclosure of further genes, peptides and proteins involved therein.

Summary of the invention

The present invention provides a significant step forward regarding the understanding of the above described pathway. By a combination of cDNA library and RACE analysis a novel human *patched*-like gene (PTCH2) has been cloned and sequenced. Several alternatively spliced mRNA forms of PTCH2 have been ideintified, including transcripts lacking segments thought to be involved in sonic hedgehog (SHH) binding and mRNAs with differentially defined 3' terminal exons. Accordingly, the invention relates to isolated such mRNAs as well as to cDNAs complementary thereto.

Brief description of the drawings

Figure 1 shows the genomic sequence of SEQ ID NO:5, wherein exons and introns are designated in the genomic sequence of the novel human patched 2 gene.

Figure 2A discloses an amino acid sequence comparison of the human PTCH2 (upper lines) and PTCH1 (lower lines) sequences.

Figure 2B is a representation of the alternative splicing events that result in different C-termini.

Figure 2C is a representation of the different variations of spliced transcripts encompassing exon 1 and exon 2 sequences.

Figure 3A is a dark-field photomicrograph of a BCC tumor hybridised with ^{35}S-labeled antisense probe showing abundant signal for PTCH1 mRNA (light grains) in all BCC tumor cells.

Figure 3B discloses PTCH2 mRNA overexpression in BCC and is in contrast mainly expressed in the basaloid cells in the periphery of the tumor nests.
Figure 3C is another BCC showing a strong PTCH2 mRNA signal in the periphery of the tumor nest (Tu), wheras no signal is detected in epidermis (Ep).
Figure 3D are sections of the same tumor (C) hybridised with the PTCH2 sense probe showed no signal.
Figure 3E shows immunoreactivity for Ki-67.
Figure 3F discloses how tumor nests under high power magnification demonstrate abundant PTCH2 mRNA signal (black grains) in the dark basaloid tumor cells and lower signal in the center (arrow).

Definitions

The terms "polypeptide", "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical analogue of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers.

The terms "isolated" "purified" or "biologically pure" refer to material which is substantially or essentially free from components which normally accompany it as found in its native state.

The term "nucleic acid" refers to a deoxyribonucleotide or ribonucleotide polymer in either single- or double-stranded form, and unless otherwise limited, encompasses known analogs of natural nucleotides that can function in a similar manner as naturally occurring nucleotides.

A "label" is a composition detectable by spectroscopic, photochemical, biochemical, immunochemical, or chemical means. For example, useful labels include ^{32}P, fluorescent dyes, electron-dense reagents, enzymes (*e.g.*, as sommonly used in a ELISA), biotin, dioxigenin, or haptens and proteins for which antisera or mono-

clonal antibodies are available (*e.g.*, the peptide of SEQ ID NO:1 can be made detectable, *e.g.*, by incorporating a radio-label into the peptide, and used to detect antibodies specifically reactive with the peptide).

As used herein a "nucleic acid probe" is defined as a nucleic acid capable of binding to a target nucleic acid of complementary sequence through one or more types of chemical bonds, usually through complementary base pairing, usually through hydrogen bond formation. As used herein, a probe may include natural (*i.e.* A, G, C, or T) or modified bases (7-deazaguanosine, inosine, *etc.*) In addition, the bases in a probe may be joined by a linkage other than a phosphodiester bond, so long as it does not interfere with hybridisation. Thus, for example, probes may be peptide nucleic acids in which the constituent bases are joined by peptide bonds rather that phosphodiester linkages. It will be understood by one of skill in the art that probes may bind target sequences lacking complete complementarity with the probe sequence depending upon the stringency of the hybridisation conditions. The probes are preferably directly labeled as with isotopes, chromophores, lumiphore, chromogens, or indirectly labeled such as with biotin to which a streptavidin complex may later bind. By assaying for the presence or absence of the probe, one can detect the presence or absence of the selct sequence or subsequence.

A "labeled nucleic acid probe" is a nucleic acid probe that is bound, either covalently, through a linker, or through ionic, van der Waals or hydrogen bonds to a label such that the presence of the probe may be detected by detecting the presence of the label bound to the probe.

The term "target nucleic acid" refers to a nucleic acid (often derived from a biological sample), to which a nucelic acid probe is designed to specifically hybridise. It is either the presence or absence of the target nucleic acid that is to be detected, or the amount of the target nucleic acid that is to be quantified. The target nucleic acid has a sequence that is complementary to the nucleic acid sequence of the corresponding probe directed to the target. The term target nucleic acid may refer to the specific

subsequence of a larger nucleic acid to which the probe is directed or to the ovarall sequence (*e.g.*, gene or mRNA) whose expression level it is desired to detect. The difference in usage will be apparent from context.

The term "recombinant" when used with reference to a cell, or nucleic acid, or vector, indicates that the cell, or nucleic acid, or vector, has been modified by the introduction of a heterologous nucleic acid or the alteration of a native nucleic acid, or that the cell is derived from a cell so modified.

The term "identical" in the context of two nucleic acids or polypeptide sequences refers to the residues in the two sequences which are the same when aligned for maximum correspondence. Optimal alignment of sequences for comparison can be conducted, *e.g.*, by the local homology algorith of Smith and Waterman (1981) *Adv. Appl. Math.* 2: 482, by the homology alignment algorithm of Needleman and Wunsch (1970) *J. Mol. Biol.* 48:443, by the search for similarity method of Pearson and Lipman (1988) *Proc. Natl. Acad. Sci.* USA 85: 2444, by computerized implementations of these algorithms (GAP, GESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI) or by inspection. The BLAST algorithm performs a statistical analysis of the similarity between two sequences; *see e.g.*, Karlin and Altschul (1993) Proc. *Nat'l Acad. Sci. USA* 90: 5873-5787.

The term "substantial identity" or "substantial similarity" in the context of a polypeptide indicates that a polypeptides comprises a sequence with at least 70% sequence identity to a reference sequence, or preferably 80%, or more preferably 85% sequence identity to the reference sequence, or most preferably 90% identity over a comparison window of about 10-20 amino acid residues. An indication that two polypeptide sequences are substantially identical is that one peptide is immunologically reactive with antibodies raised against the second peptide. Thus, a polypeptide is substantially identical to a second polypeptide, for example, where the two peptides differ only by a conservative substitution.

An indication that two nucleic acid sequences are substantially identical is that the polypeptide which the first nucleic acid-encodes is immunologically cross reactive with the polypeptide encoded by the second nucleic acid. Another indication that two nucleic acid sequences are substantially identical is that the two molecules hybridise to each other under stringent conditions.

The phrase "hybridising specifically to", refers to the binding, duplexing, or hybridising of a molecule only to a particular nucleotide sequence under stringent conditions when that sequence is present in a complex mixture (*e.g.*, total cellular) DNA or RNA. The term "stringent conditions" refers to conditions under which a probe will hybridise to its target subsequence, but to no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridise specifically at higher temperatures. Generally, stringent conditions are selected to be about 5°C lower than the thermal melting point ™ for the specific sequence at a defined ionic strength and pH. The Tm is the temperature (under defined ionic strength, pH, and nucleic acid concentration) at which 50% of the probes complementary to the target sequence hybridise to the target sequence at equilibrium. (As the target sequences are generally present in excess, at Tm, 50% of the probes are occupies at equilibrium). Typically, stringent conditions will be those in which the salt concentration is less than about 1.0 M Na ion, typically about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for whort probes (*e.g.*, 10 to 50 nucleotides) and at least about 60°C for long probes (*e.g.*, greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide.

The term "antibody" refers to a polypeptide substantially encoded by an immunoglobulin gene or immunoglobulin genes, or fragments thereof which specifically bind and recognize an analyte (antigen).

A "chimeric antibody" is an antibody molecule in which (a) the constant region, or a portion thereof, is altered, replaced or exchanged so that the antigen binding site (variable region) is linked to a constant region of a different or altered class, effector function and/or species, or an entirely different molecule which confers new properties to the chimeric antibody, *e.g.*, an enzyme, toxin, hormone, growth factor, drug, *etc.*; or (b) the variable region, or a portion thereof, is altered, replaced or exchanged with a variable region having a different or altered antigen specificity.

The term "immunoassay" is an assay that utilizes an antibody to specifically bind an analyte. The immunoassay is characterized by the use of specific binding properties of a particular antibody to isolate, target, and/or quantify the analyte.

The phrases "specifically binds to a protein" or "specifically immunoreactive with", when referring to an antibody refers to a binding reaction which is determinative of the presence of the protein in the presence of a heterogeneous population of proteins and other biologics. Thus, under designated immunoassay conditions, the specified antibodies bind preferentially to a particular protein and do not bind in a significant amount to other proteins present in the sample. Specific binding to a protein under such conditions requires an antibody that is selected for its specificity for a particular protein. A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select monoclonal antoibodies specifically immunoreactive with a protein. *See* harlow and Lane (1988) *Antibodies, A Laboratory Manual,* Cold Spring Harbour Publications, New York, for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity.

A "gene product", as used herein, refers to a nucleic acid whose presence, absence, quantity, or nucleic acid sequence is indicative of a presence, absence, quantity, or nucleic acid composition of the gene. Gene products thus include, but are not limited to, and mRNA transcript acDNA reverse transcribed from an mRNA, and RNA

transcribed from that cDNA, a DNA amplified from the cDNA, an RNA transcribed from the amplified DNA or subsequences of any of these nucleic acids. Polypeptides expressed by the gene or subsequences thereof are also gene products. The particular type of gene product will be evident from the context of the usage of the term.

A "modified drug" means a compound, which retains the pharmaceutical properties of the original drug or active substance while the structure thereof has been modified. Further, encompassed by the term "drug" are also compounds useful in diagnostic methods by their specific binding properties.

Detailed description of the invention

In a first aspect, the present invention relates to an isolated human protein, or an analogue or a variant thereof, capable of participating in the human PTCH/SHH pathway during embryonic development and/or carcinogenesis, such as basal cell carcinoma. The novel protein according to the invention is encoded by a novel gene, which isolated nucleic acid is described in detail below and which is denoted *patched 2* (PTCH2) due to its similarities with *patched 1* (PTCH1). Accordingly, the protein according to the invention exhibits substantial differences in sequence and functions when compared to human PTCH1protein. The protein according to the invention is best characterized by its functions which when compared to human PTCH1 are similar but distinct therefrom in certain ways, more specifically disclosed below in the section "Results and discussion". The novel human PTCH2 protein according to the invention is also distinct from the previously isolated mouse PTCH2. Thus, in the preferred embodiment thereof, it comprises a substantial part of the amino acid sequence disclosed in SEQ ID NO: 1 and submitted to the GenBank under protein id no AAD17260.1. even though it is to be understood that the present invention encompasses any fragment, analogue or variant thereof exhibiting the biological functions of the PTCH2 protein disclosed herein. Thus, preferably, the present protein comprises at least about 1000, more preferably at least about

1040 and most preferably essentially all of the amino acids of the sequence denoted SEQ ID NO: 1, such as about 1100.

The proteins according to the invention are easily prepared by someone skilled in this field by recombinant DNA techniques using the molecules disclosed below or any synthetic method (*see e.g.* Barany and Merrifield, Solid-Phase Peptide synthesis, pp. 3-284 in The Peptides: Analysis, Synthesis, Biology, Vol. 2: Special Methods in Peptide synthesis, Part A, Merrifield *et al.*, J. Am. Chem. Soc., 2149-2156).

The present invention also relates to the use of the peptides, polypeptides and proteins disclosed herein as lead compounds in methods aimed at finding novel substances, i.e. modified drugs, such as substances exhibiting equivalent or even more advantageous properties than the lead compounds as such. Such modified drugs may also be designed by methods of combinatorial chemistry, wherein a structurally similar compound is specifically designed e.g. by aid of computers. Alternatively, the present modified drug is identidied by screening of a library of candidate compounds, e.g. using an antibody according to the invention. In the present context, it is to be understood that when such a modified drug has been identified, it is possible to produce it by any other suitable technique. The invention also relates to proteomic methods wherein the present molecules are used as well as to such a use *per se*.

A second aspect of the present invention is a nucleic acid encoding a protein, an analogue or a variant thereof as defined above, that is, the protein coding region of the novel human isolated PTCH2 gene. The PTCH2 gene is 57% identical to PTCH1 and 91% identical to the published mouse Ptch2 sequence (*see* Motoyama *et al.*, (1998), supra). Thus, preferably, the nucleic acid according to the present invention comprises at least about 3000 bases, more preferably at least about 3094 bases and most preferably essentially all of the sequence denoted SEQ ID NO: 2.

In a specific aspect, the present invention relates to the isolated human genomic PTCH2 nucleic acid comprising parts or all of the genomic sequence denoted SEQ ID NO: 5. In the disclosure of the genomic sequence shown in Fig 1, the exon/intron structure of the present gene is shown. Further to the exons shown therein, exon 12a and 12b has also been identified, as specifically defined by SEQ ID NO:3 and SEQ ID NO:4, respectively. Interestingly, there is a splice variant that joins exon 12a to a 3' segment of exon 12b with conservation of the intronic GT-AG dinucleotides. Exons 12a and 12b are not variants, but the actual exons of the gene identified by sequencing the corresponding genomic region. (Materials and methods were as discribed beloow). Accordingly, these findings show that PTCH2 has the same intron/exon structure organization as PTCH1. In another embodiment of this aspect, the present invention relates to a transcript that has skipped only one of the exons 9 and 10 defined in Fig 1. In an alternative embodiment, the transcript according to the invention has skipped both of exon 9 and 10. The splice variants of the present gene are discussed in more detail below in the section "Results", all of which are included within the scope of the present invention. This aspect of the invention advantageously enables design of suitable PCR primers, which in turn enables screening for mutations of all of the coding sections thereof, *e.g.* by SSCP analysis, sequencing, or any other suitable method known to someone skilled in this field. Thus, the novel human PTCH2 gene according to the invention has been localized by radiation hybrid mapping to chromosome 1p32-35 with D1S211 and WI-1404 as closest flanking markers and with an estimated localization 5.5cR from D1S443. This region is often lost by LOH in various different tumor types, such as neuroblastoma, melanoma, breast cancer, colon cancer *etc.* Accordingly, PTCH2 is a candidate for a tumor suppressor gene in this region and the present invention also encompass diagnostic methods based on this new disclosure.

To this chromosomal region, three cancer predisposition syndromes have also been mapped, namely, familial melanoma CMM1, modifier locus for familial adenomatous polyposis hMom1 and Michelin Tire Baby Syndrome. PTCH2 is further a candidate for the gene behind these heritary syndromes. The present molecules are the-

refore advantageously used in the context of these conditions, *e.g.* in therapy and/or diagnosis, such as in assays.

Further, the invention also relates to various PCR primers based on intronic sequences, allowing amplification of all coding sequence. Such primers are advantageously used for mutation screening.

Further, the present invention also relates to the any isolated nucleic acid capable of specifically hybridising to a nucleic acid according to the invention. In addition, the invention also relates to such an isolated nucleic acid which comprises one o more mutations compared to the genomic sequence as well as the use of the novel isolated nucleic acids, *e.g.* to identify mutations for diagnostic and/or therapeutic purposes.

Further embodiments of this aspect of the invention includes nucleic acid probes, *e.g.* DNA probes, labelled nucleic acids, cDNAs, RNAs *etc.*, that is, all gene products obtainable by someone skilled in this field based on the novel isolated human PTCH2 gene.

Another aspect of the invention is a nucleic acid corresponding to any one of the splicing variants disclosed in Figure 2B , a protein or polypeptide encoded thereof as well as various uses thereof.

As regards the preparation of nucleic acids according to the invention, any suitable recombinant DNA technique or synthetic method may be used. (For general laboratory procedures useful in this context, *see e.g.* Sambrook *et al.*, Molecular Cloning, A Laboratory Manual, 2nd ed., Vol. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989; Berger and Kimmel, Guide to Molecular Cloning Techniques, Methods in Enzymology, Vol. 153, Academic Press, Inc., San Diego, CA; Current Protocols in Molecular Biology, F.M. Ausbel *et al.*, eds., Current Protocols (1994)).

A further aspect of the present invention is a vector comprising a nucleic acid as defined above. Vectors are *e.g.* useful for transforming cells in vitro or in vivo to express the proteins and peptides according to the invention and may e.g be plasmids, viruses *etc.*

Another aspect of the invention is a recombinant cell, such as a eucaryotic, *e.g.* a mammalian cell, or a procaryotic cell, *e.g.* a bacteria, comprising a vector as defined above. Such cells may *e.g.* be used to monitor expression levels of the proteins and polypeptides according to the invention in a wide variety of contexts. For example, when the effects of a drug is to be determined, the drug will be administered to the transformed organism, tissue or cell. Accordingly, model systems including such cells are another aspect of the invention.

A further aspect of the invention is an antibody, such as a monoclonal or polyclonal antibody, which specifically binds to a protein or polypeptide according to the invention. An exemplary immunoglobulin (antibody) structural unit comprises a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kD) and one "heavy" chain (about 50-70 kD). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The terms variable light chain (V_L) and variable haeavy chain (V_H) refer to these light and heavy chains, respectively.

The invention also encompasses chimeric or other antibodies that binds the present proteins or polypeptides. Further, the invention also relates to the use of the present antibodies in assays. (In this context, *see e.g. Fundamental Immunology*, Third Edition, W.E. Paul, ed., Raven Press, N.Y. 1993).

Further, the invention also relates to a recombinant cell expressing an antibody according to the invention.

In general, prokaryotes can be used for cloning the DNA sequences encoding a human anti-PTCH2 immunoglobulin chain. *E. coli* is one prokaryotic host particularly useful for cloning the DNA sequences of the present invention. Microbes, such as yeast are also useful for expression. *Saccharomyces* is a preferred yeast host, with suitable vectors having expression control sequences, an origin of replication, termination sequences and the like as desired. Typical promoters include 3-phosphoglycerate kinase and other glycolytic enzymes. Inducible yeast promoters include, among others, promoters from alcohol dehydrogenase 2, isocytochrome C, and enzymes responsible for maltose and galactose utilization.

Mammalian cells are a particularly preferred host for expressing nucleotide segments encoding immunoglobulins or fragments thereof (*see, e.g.* Winnacker, *From Genes to Clones,* VCH Publishers, N.Y., 1987). A number of suitable host cell lines capable of secreting intact heterologous proteins have been developed in the art, and include CHO cell lines, various COS cell lines, HeLa cells, L cells and myeloma cell lines. Preferably, the cells are nonhuman. Expression vectors for these cells can include expression control sequences, such as an origin of replication , a promoter, an enhancer (Queen *et al.* (1986) *Immunol. Rev.* 89:49), and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences. Preferred expression control sequences are promoters derived from endogenous genes, cytomegalovirus, SV40, adenovirus, bovine pappillomavirus, and the like (*see, e.g.*, Co *et al.* (1992) *J. Immunol.* 1458: 1149).

An additional aspect of the present invention is a kit for the detection of a human PTCH2 gene or polypeptide comprising in a container a molecule selected from the group consisting of a nucleic acid, a polypeptide or a protein or an antibody according to the invention. Further suitable components of such a kit are easily determined by someone skilled in this field as are the conditions for the use thereof.

Further, the invention also realtes to the use of a nucleic acid selected from the group consisting of SEQ ID NOS: 2-4 and SEQ ID NO: 5 in gene therapy. In addition to said specifically disclosed sequences, any one of the herein disclosed exons may be used to this end.For a review of gene therapy procedures, *see* Anderson, *Science* (1992) 256:808-813; Nabel and Felgner (1993) *TIBTECH* 11: 211-217; Mitani and Caskey (1993) *TIBTECH* 11: 162-166; Mulligan (1993) *Science* 926-932; Dillon (1993) *TIBTECH* 11: 167-175; Miller (1992) *Nature* 357: 455-460; Van Brunt (1988) *Biotechnology* 6(10): 1149-1154; Vigne (1995) *Restorative Neurology and Neuroscience* 8: 35-36; Kremer and Perricaudet (1995) *British Medical Bulletin* 51(1) 31-44; Haddada *et al.* (1995) in *Current Topics in Microbiology and Immunology* Doerfler and Böhm (eds) Springer-Verlag, Heidelberg Germany; and Yu *et al.*, *Gene Theraphy*(1994) 1:13-26.

Delivery of the gene or genetic material into the cell is the first critical step in gene therapy treatment of disease. A large number of delivery methods are well known to those of skill in the art. Such methods include, for example liposome-based gene delivery (Debs and Zhu (1993) WO 93/24640; Mannino and Gould-Fogerite (1988) *BioTechniques* 6(7): 682-691; Rose U.S. Pat No. 5,279,833; Brigham (1991) WO 91/06309; and Felgner *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84: 7413-7414), and replication-defective retroviral vectors harboring a therapeutic polynucleotide uence as part of the retroviral genome (*see, e.g.*, Miller *et al.* (1990) *Mol. Cell. Biol.* 10:4239 (1990; Kolberg (1992) *J. NIH Res.* 4:43, and Cornetta *et al. Hum. Gene Ther.* 2:215 (1991)). Widely used retroviral vectors include those based upon nurine leukemia virus (MuLV), gibbon ape leukemia virus (GaLV), Simian Immuno deficiency virus (SIV), human immuno deficiency virus (HIV), and combinations thereof. *See, e.g.*, Buchscher *et al.* (1992) *J. Virol.* 66 (5) 2731-2739; Johann *et al.* (1992) *J. Virol.* 66 (5):1635-1640 (1992); Sommerfelt *et al.*, (1990) *Virol.* 176:58-59; Wilson *et al.* (1989) *J. Virol.* 63:2374-2378; Miller *et al.*, *J Virol.* 65:2220-2224 (1991); Wong-Staal *et al.*, PCT/US94/05700, and Rosenburg and Fauci (1993) in *Fundamental Immunology. Third Edition* Paul (ed) Raven Press, Ltd., New York and the references therein, and Yu *et al.*, *Gene Therapy* (1994) supra).

The present invention may also be used in the pharmaceutical industry. For example, it will provide information that eventually may enable cells from fetal tissue, which may the be transplanted into patients suffering from *e.g.* Parkinson's disease or cancer, such as BCC. (For a brief review of methods of drug delivery, *see* Langer 249:1 527-1533 (1990), Remington's Pharmaceutical Sciences, Mack Publishing Company, Philadelphia, PA, 17th ed. (1985) *etc.*)

Detailed description of the drawings

Figure 1 shows the genomic sequence of SEQ ID NO:5, wherein exons and introns are designated in the genomic sequence of the present human patched 2 gene. However, exons 12a and 12b discussed above are not specifically shown in Figure 1, but is instead disclosed as the separate sequences SEQ ID NO:3 and SEQ ID NO:4, respectively. Figure 2A discloses an amino acid sequence comparison of the human PTCH2 (upper lines) and PTCH1 (lower lines) sequences. Vertical lines indicate identical amino acids, while dots similar amino acids. The PTCH2 sequence presented is composed of the original cDNA clones and of the products of the 5' RACE analysis.

Figure 2B is a representation of the alternative splicing events that result in different C-termini. In the parotid gland and the colon, the penultimate and the last exon are canonically joined together. In fetal brain however the penultimate exon with part of the 3' intron functions as the terminal exon. The intronic sequence is shown by small letters with the flanking exonic by capital letters. Above the nucleotide sequence, the deduced amino acid sequence is shown, and below is the corresponding sequence of the mouse Ptch2. The conserved intronic dinucleotides are shown by bold letters and the termination signals are indicated by asterisks. Note the absence of conservation of the position of the termination codons between the mouse and human PTCH2 sequences. The putative polyadenylation signals are also shown in this diagram. The genomic organization was obtained by analyzing BAC clones encompassing the PTCH2 gene.

Figure 2C is a representation of the different variations of spliced transcripts encompassing exon 1 and exon 2 sequences. The canonical exons 1 and 2 are shown by boxes and the intron between them by a solid line. The GT and AG dinucleotides spanning the sequences that are used as introns in individual transcripts are indicated by small letters. G, Genomic structure, derived from sequencing segements of BAC clones encompassing the PTCH2 gene; C, Canonical transcript; A, Transcript A (the skipped exons 9 and 10 of this product are not shown in the diagram); B, Transcript B.

Figure 3A is a dark-field photomicrograph of a BCC tumor hybridised with ^{35}S-labeled antisense probe showing abundant signal for PTCH1 mRNA (light grains) in all BCC tumor cells.

Figure 3B discloses PTCH2 mRNA overexpression in BCC and is in contrast mainly expressed in the basaloid cells in the periphery of the tumor nests.

Figure 3C is another BCC showing a strong PTCH2 mRNA signal in the periphery of the tumor nest (Tu), wheras no signal is detected in epidermis (Ep).

Figure 3D are sections of the same tumor (C) hybridised with the PTCH2 sense probe showed no signal.

Figure 3E shows immunoreactivity for Ki-67 (brown precipitate) seen in the periphery, in the cells that showed strong upregulation of PTCH2 mRNA.

Figure 3F discloses tumor nests under high power magnification demonstrate abundant PATCH2 mRNA signal (black grains) in the dark basaloid tumor cells and lower signal in the center (arrow). Bars (A-E), 24 μm, and F, 6 μm.

EXPERIMENTAL

Materials and methods

In the present context, a general reference is made to G. Zaphiropoulos et al., Cancer Res., vol. 59, p. 787-792, February 15, 1999, disclosing useful methods in the present context. All references mentioned in the present application are hereby included herein by reference. The examples below are not intended to limit the scope of the invention but merely as an illustration.

The RACE analysis was performed essentially as described before (Zaphiropoulos, P.G. and Toftgård, R. (1996): "cDNA cloning of a novel WD repeat protein mapping to the 9q22.3 chromosomal region", DNA Cell Biol. 15, 1049-1056) using the Marathon kit (Promega). The primer sequences used for RACE are available upon request.

The PTCH2, 35S-labeled RNA probes used for the in situ hybridisations, that were performed as previously described (Undén *et al.*, (1997), supra), corresponded to positions 218 to 437 and 838 to 920 in the PTCH2 sequence of SEQ ID NO:1 .

Results and discussion

In order to identify additional components of the PTCH/SHH cascade of signalling events, the Incyte LifeSeq™ database (Incyte Pharmaceuticals Inc., Palo Alto, CA, USA) was searched using PTCH sequences. In addition to clones representing the PTCH cDNA, two nearly identical cDNAs were identified, from the parotid gland and the colon, that contained sequences similar to, but distinct from, the 3' end of PTCH. By 5' RACE analysis using fetal brain cDNAs additional sequence information from these transcripts (termed PTCH2) and corresponding to a full length cDNA, was obtained (Fig. 2A). PTCH2 is 57% identical to PTCH1, with a significantly variable region present between the transmembrane domains 6 and 7, and 91% identical to the recently published mouse Ptch2 sequence (Motoyama, J., Takabatake, T., Takeshima, K. and Hui, C. (1998): "Ptch2, a second mouse Patched gene is co-expressed with Sonic hedgehog", Nature Genet. 18, 104-106). In simila-

rity with the mouse gene, PTCH2 lacks the C-terminal extension present in human, mouse and chicken PTCH1 (Goodrich, L.V., Johnson, R.L., Milenkovic, L., McMahon, J.A., and Scott, M.P. (1996): "Conservation of the hedgehog/patched signalling pathway from flies to mice: Induction of a mouse patched gene by Hedgehog", Genes Dev. **10**, 301-312, Marigo, V., Scott, M.P., Johnson, R.L., Goodrich, L.V. and Tabin, C.J. (1996): "Conservation in hedgehog signalling: Induction of a chicken patched homolog by Sonic hedgehog in the developing limb", Development **122**, 1225-1233). However, according to the present invention, it has been shown that the human PTCH2 cDNA terminates 36 amino acids earlier that the mouse Ptch2 sequence. Moreover, when 3' RACE was perfomed from fetal brain, an alternate C-terminal region was identified. This had a high structural similarity with the mouse Ptch2 C-terminal sequence and originates from the genomic region that links the last two exons of PTCH2 (Fig. 2B). Therefore, in these alternatively spliced transcripts, the penultimate exon with a segment of the contiguous 3' intron serves as the terminal exon.

Moreover the human and mouse transcripts differed in the position of the termination signals (the human sequence is 21 amino acids longer), suggesting a non-conserved, species-specific function of this alternate C-terminal domain. The finding of two possible C-terminal regions for PTCH2 is intriguing and implies a role of this phenomenon in modulating signalling. Additional alternatively spliced transcripts were also identified by the RACE analysis (Fig. 2C). Transcript A lacks the sequence that corresponds to exons 9 and 10 of PTCH1 (preliminary comparisons of the intron-exon junctions of PTCH2 with PTCH1 indicate a similar genomic organization), with the open reading frame being retained at the exon 8 to exon 11 junction. Exons 9 and 10 code for the last part of the first extracellular loop and for transmembrane domains 2 and 3 in the putative structure of the PTCH1 protein. Furthermore this transcript also lacks a 5' segment of the canonical exon 2, due to the use of an alternative 3' splice site present in this exon, with the open reading frame being maintained. The functional consequence of this alternative splicing is not yet known, but it is interesting to note that the extracellular loops in PTCH1 are

presumed to be involved in binding of the ligand SHH (Marigo *et al.*, (1996), Nature 384, supra; Stone *et al.*, (1996), Nature 384, supra) and that insertion of a neocassette in intron 9 of of the mouse PTCH1 gene is associated with a severe phenotype (Hahn, H., Wojnowski, L., Zimmer, A.M., Hall, J., Miller, G. and Zimmer, A. (1998): "Rhabdomyosarcomas and radiation hypersensitivity in a mouse model of Gorlin syndrome", Nature Med. 4, 619-622). Furthermore, exons 9 and 10 encode part of a putative sterol sensing domain (Osborne, T.F. and Rosenfeld, J.M. (1998): "Related membrane domains in proteins of sterol sensing and cell signalling provide a glimpse of treasures still buried within the dynamic realm of intracellular metabolic regulation", Curr. Opin. Lipidol. 9, 137-140, also found in PTCH1, and which has recently been implicated in mediating the potent modulating effect of cholesterol on SHH/PTCH signalling (Cooper, M.K., Porter, J.A., Young, K.E., and Beachy, P.A. (1998): "Teratogen-mediated inhibition of target tissue response to Shh signalling", Science 280, 1603-1607). Thus, if PTCH2 also serves as a receptor for SHH and/or related factors, the receptor form lacking exons 9 and 10 may show altered signalling properties. Transcript B contains additional sequences between canonical exons 1 and 2, that originate from the 5' end of intron 1. The open reading frame that includes the initiator methionine of exon 1 is not maintained in this transcript, suggesting that, if this transcript is functional, either the methionine in exon 2 or non-methionine codons are used in order to produce a protein product, in similarity to what has been proposed for the alternative spliced products of human PTCH1 (Hahn *et al.*, Cell 85, supra). By radiation hybrid mapping the PTCH2 gene was localized to the short arm of chromosome 1, in difference to PTCH1 residing on chromosome 9q22.3.

The mouse and zebrafish homologs of PTCH2 have been reported to be expressed in a partly overlapping pattern with PTCH1 during embryonic development and to be induced by SHH (Motoyama *et al.*, (1998) Nature Genet. 18, supra, Concordet, J.P., Lewis, K.E., Moore, J.W., Goodrich, L.V., Johnson, R.L., Scott, M.P., and Ingham, P.W. (1996): "Spatial regulation of a zebrafish patched homologue reflects

the roles of sonic hedgehog and protein kinase A in a neural tube and somite patterning", Development 122, 2835-2846), implicating a role in this signalling pathway. We were with this background interested to analyze the expression of PTCH2 in BCCs which show consistent upregulation of PTCH1 in all tumor cells (Undén *et al.*, (1997) Cancer res. 57, supra). In situ hybridisation was performed on six familial and four sporadic BCCs of different histological subtypes. A strong positive signal for PTCH2 mRNA was observed exclusively in the tumor cells of all BCCs. Notably, the signal was consistently stronger in the palisading peripheral cells of the tumor nests (Fig. 2). These cells also showed a positive immunostaining for the cell proliferation marker, Ki-67.

The finding that in BCCs having frequent mutations in the PTCH1 gene, the expression of the PTCH2 mRNAs is upregulated, tightly links the novel PTCH2 according to the invention with the PTCH/SHH cascade of signalling events. It is therefore likely that PTCH2 represents a target gene of this pathway which is under the negative regulation of PTCH1, precisely as PTCH1 itself. Moreover this observation strongly suggests that PTCH2 has functions distinct from PTCH1 since upregulation of PTCH2 expression appears unable to compensate for inactive PTCH1 protein. This conclusion is also supported by the early embryonic lethality seen in PTCH1 (-/-) mice 5,13) and the lack of genetic heterogeneity in Gorlin syndrome. However, whether PTCH2 may block the constitutive signalling of SMO, or could act as an additional SHH receptor, possible dependent on alternative splicing, remains as the subject of further experimentation.

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AMENDED CLAIMS

1. An isolated human protein or an analogue or variant thereof capable of participating in the human PTCH/SHH pathway during embryonic development and/or carcinogenesis, which is essentially comprised of SEQ ID NO: 1.
2. A nucleic acid encoding a protein according to claim 1.
3. An isolated variant of the nucleic acid according to claim 2.
4. An isolated nucleic acid capable of specifically hybridising to a nucleic acid according to claim 2 or 3.
5. A protein according to claim 1 or a nucleic acid according to any one of claims 2-4 for use as a medicament.
6. Use of a protein according to claim 1 or a nucleic acid according to any one of claims 2-4 in the manufacture of a medicament for the treatment of a condition involving tumors, such as BSS.
7. A method of in vitro or in vivo diagnosis, wherein a protein according to claim 1 or a nucleic acid according to any one of claims 2-4 is used.
8. A method of screening wherein a library of suitable candidate compounds is screened for modified drugs using a protein according to claim 1 as a lead compound.
9. A method of synthesis of a modified drug, wherein a protein according to claim 1 is used.
10. A modified drug identified by the method according to claim 8 or synthesized according to claim 9.
11. A vector comprising a nucleic acid according to any one of claim 2-4.
12. A recombinant cell comprising a vector according to claim 11.
13. An antibody which specifically binds to a protein according to claim 1.
14. A recombinant cell expressing an antibody according to claim 13.
15. A kit for the detection of a human PTCH2 gene or polypeptide comprising in a container a molecule selected from the group consisting of a nucleic acid according to any one of claims 2-4, a protein according to claim 1 or an antibody according to claim 13.
16. Use of a nucleic acid according to any one of claims 2-4 in gene therapy.

17. Use of nucleic acid according to any one of claims 2-4 as a probe, a primer or a diagnostic reagent.

The intron sequences between exons 2 - 3 and exons 18 - 19 are missing (introns: small letters, exons: capital letters). Small letters in the first exon indicate nucleotides that have not been unambigouisly determined.

Exon 1

```
   1 CGGGTGAATC CCGGCGCCGC GCCCCGGACC CGCAGCTCCC TGCACTCCTC
  51 CCTCCCAGCC GCTTTAACAC CCACACCCCA CAGTCTCTCC CACGsCCGCG
 101 CCTTGGCGGC CCCACTGAAT CCCTACGCGG GGCCCAGCGG TACCGGGAGA
 151 CCGGGCTAGC CTATGGGAGC GCCCAGATAA CGCGGGTTGG GGGCGCCCGC
 201 GCCCcCATCC CCGCCAGCAT GACTCGATCG CCGCCCCTCA GAGAGCTGCC
 251 CCCGAGTTAC ACACCCCCAG CTCGAACCGC AGCACCCCAG gtgagtagag
 301 ggggagctgg aagaaggaag agagcggagc caggtctgtc actcgggcct
 351 ctgcaaggtt tgtgatgtct tgaagtgccg agtgtcatta gatgtctgaa
 401 ggcaagtgag agccagcacc gcaagcaagt tgtgcgtgtg tgtcggtgtg
 451 tctgtgccgg tgtctcctca tcgtctggcc agtgagaatg aatgtctgtg
 501 ggttcacctc tgtgtccacc cgacgacagg tgtgtgtaca tatgtatcct
 551 gctctcagaa aatgggccta tgccgccggg cgcggtgact cacgcctgta
 601 atcccaacac tgggaggctg aggcaggcag attacctgag gtcaggagtt
 651 cgagaccagc caggccaaca tggggaaact ctgtctctac taaaaataaa
 701 aattagcagg gcgtggtggc gggcgcctgt agtcccaact actcgggagg
 751 ctgaggcagg agaatctctt gaacctggga ggcggaggtt gcagtcaagc
 801 cgagatcaca ccactgcact ccagccaggg caacagagcg agatgcgtct
 851 caaaaaaaaa aaaaaaaaaa aaaaggagag aaaacaaaaa gaaaagaaag
 901 gaaaataggc ctatgccttc ctcaggtgtg tgctggggat ggtgggtgtt
 951 acatcttcca agtctgggcc tgtgtctgtg ttggtgctcc ctgtcccaca
1001 tccagaaatc aagaagcgag ggctgggcag cagatataca gggtgagaag
```

Fig. 1

```
1051  ggaaggattt catgcattgt tacagtgatg cctggctgac ccttctcttt
                EXON 2
1101  ccatcccagA TCCTAGCTGG GAGCCTGAAG GCTCCACTCT GGCTTCGTGC
1151  TTACTTCCAG GGCCTGCTCT TCTCTCTGGG ATGCGGGATC CAGAGACATT
1201  GTGGCAAAGT GCTCTTTCTG GGACTGTTGG CCTTTGGGGC CCTGGCATTA
1251  GGTCTCCGCA TGGCCATTAT TGAGACAAAC TTGGAACAGC TCTGGGTAGA
1301  AGTGGGCAGC CGGGTGAGCC AGGAGCTGCA TTACACCAAG GAGAAGCTGG
1351  GGGAGGAGGC TGCATACACC TCTCAGATGC TGATACAGAC CGCACGCCAG
1401  GAGGGAGAGA ACATCCTCAC ACCCGAAGCA CTTGGCCTCC ACCTCCAGGC
1451  AGCCCTCACT GCCAGTAAAG TCCAAGTATC ACTCTATGGG AAG.......
1501  .......... .......... .......... .......... .........g
1551  tgagtctggc tgagcccctg agcagctggg ggcgaggcgt gctgtggggg
1601  ttctggagtg ggaatcccct tcttctgctg atctcctatg ccccctggcta
            EXON 4
1651  ttgcagTCCT GGGATTTGAA CAAAATCTGC TACAAGTCAG GAGTTCCCCT
1701  TATTGAAAAT GGAATGATTG AGCGGgtaag tgtcctgaga gggagtagag
1751  gcagaacttt ttctgtagcg tgggaggact cagagaccga gcaagcccca
1801  cagcctgcaa tctgcccccc taaaactaag gagggggatt gcagagggca
1851  tcctacaaag gttgtggggc aggactgacg tggcccgggg tatccctggc
        EXON 5
1901  agATGATTGA GAAGCTGTTT CCGTGCGTGA TCCTCACCCC CCTCGACTGC
1951  TTCTGGGAGG GAGCCAAACT CCAAGGGGGC TCCGCCTACC TGCCgtgagt
2001  gccactcctg gggccctgct tcatctcccg ctggggactc tcccagcaga
2051  aaggaggggt ctggggaatg aggatgatca aaaccttacc aaggtcctaa
2101  ttacctccca ggccaggaac agagagcatg ggcttcccca aggctctctc
2151  cacatcctcc ttctctttcc ctctcaagga aggaagacct gacttattta
2201  cacaaaacta aacacaaaga tctgtaagat ctgagcaaag gagaaaaaga
2251  tccccacaaa gaggctttgc tgggggaaat tacctaggtg tttgctaagc
2301  cattgcccag gccagaaaga aaacctgcta caggcatgtg cctgctggtt
2351  gtatattaga accaagcaca cagcttggta aggaactcag tggggccttt
```

Fig. 1 (cont.)

```
2401  ctgggccctt tctatgtatt aggtaaccct gccctgatat tcgtctcagc
2451  cccttgtact cttctacagc tcactgtagc accctggtgg gcccatgcag
2501  cctggcagtt ctgagaagct gaggcttgca caccctccat atggaaggac
2551  aaatcggcag ataagaggag ggtggggtac agcatggcgc cccagcagca
2601  gtttggagcc tgggtttttcg tccctgaccc tcaccaacta taggctttte
                EXON 6
2651  cctcagCGGC CGCCCGGATA TCCAGTGGAC CAACCTGGAT CCAGAGCAGC
2701  TGCTGGAGGA GCTGGGTCCC TTTGCCTCCC TTGAGGGCTT CCGGGAGCTG
2751  CTAGACAAGG CACAGGTGGG CCAGGCCTAC GTGGGGCGGC CCTGTCTGCA
2801  CCCTGATGAC CTCCACTGCC CACCTAGTGC CCCCAACCAT CACAGCAGGC
2851  AGgtgggttc caaccaggtc tgccagggaa aggctgtttt ccttcccttt
2901  cccttcctca tactcctgtg ttctgggggga gctgactgct ctgtgccctg
2951  acccccccact tcctggccat tattaccctg ctcccacagt gccaggcccc
3001  caatgttcca ttcccattca gttatcctac ggagccctca agtggtatat
3051  atgaatccct ttttcctttt ctaagcctag ataaggctgg acttcttttt
3101  ttttttttt ttgagtctca ctctgtcacc caggctggag tgcagtagtt
3151  cgatcttggc tcactgcaac ctcggctcaa gcaattctcc tgccttagcc
3201  tcctgagtag ctgggattac aggtgcccac caccatgccc ggctaatttt
3251  tattagcctc ccaaagtgct gggattacag gcgtgagcca ctgcgcctgg
3301  ccaaggctgg acttttttatc aaaatagact aatacaggga aactaagaac
3351  acagcaggta agcatgaata tcatacctgg tttcccaggt ttctttgtgg
3401  ccctgcaaat gtggtacttt tttcagaatc cgccagttac accagctcct
3451  cccagaagcc tacttccagg cctctgcttc cccttggggc ttcctgtctg
3501  cgggatacta gctgttcact cctgcagagc agtcaagagg ctcagaatag
3551  ttacctacac tccagcccta ctgagcttca tggcagcgtg gttcctggag
3601  gtggaagccc agggacactc agttatccac ggccagggcc ttgagcatta
                EXON 7
3651  acccctcctg ttcccctcca gGGCTCCCAA TGTGGCTCAC GAGCTGAGTG
3701  GGGGCTGCCA TGGCTTCTCC CACAAATTCA TGCACTGGCA GGAGGAATTG
```

Fig. 1 (cont.)

```
3751  CTGCTGGGAG GCATGGCCAG AGACCCCCAA GGAGAGCTGC TGAGgtaggg
3801  tctcctctgg gagttggtga ggggactctg ttcatgagaa cccatactgt
3851  aatgccaggc agctctggca aaaggccctt cacatccctc accaggtgtt
                                                  EXON 8
3901  tgggccagct ctgacccctg gttctcccac acccccacca gGGCAGAGGC
3951  CCTGCAGAGC ACCTTCTTGC TGATGAGTCC CCGCCAGCTG TACGAGCATT
4001  TCCGGGGTGA CTATCAGACA CATGACATTG GCTGGAGTGA GGAGCAGGCC
4051  AGCACAGTGC TACAAGCCTG GCAGCGGCGC TTTGTGCAGg tcggtatgga
4101  caaggacaag gggggtgccc tgaggccatt ccctcctcct gccccctcct
                                EXON 9
4151  atccaccctg tttctccagC TGGCCCAGGA GGCCCTGCCT GAGAACGCTT
4201  CCCAGCAGAT CCATGCCTTC TCCTCCACCA CCCTGGATGA CATCCTGCAT
4251  GCGTTCTCTG AAGTCAGTGC TGCCCGTGTG GTGGGAGGCT ATCTGCTCAT
4301  Ggtgggtctt gcacctggca ccttgccccc accccacctc caaccagtgc
                                                  EXON 10
4351  ccaccctggg agcccctgag actgcccttt cccccacag CTGGCCTATG
4401  CCTGTGTGAC CATGCTGCGG TGGGACTGCG CCCAGTCCCA GGGTTCCGTG
4451  GGCCTTGCCG GGGTACTGCT GGTGGCCCTG GCGGTGGCCT CAGGCCTTGG
4501  GCTCTGTGCC CTGCTCGGCA TCACCTTCAA TGCTGCCACT ACCCAGgtac
4551  gccaggactg cagggcagac tcagtgccag tcaccaggct tcacgggtcc
                                EXON 11
4601  tcagctgccc gctcctctgc ccctccagGT GCTGCCCTTC TTGGCTCTGG
4651  GAATCGGCGT GGATGACGTA TTCCTGCTGG CGCATGCCTT CACAGAGGCT
4701  CTGCCTGGCA CCCCTCTCCA Ggtggggcct tgtcccccag ggctcatctg
4751  aggcagctca gcttactggt taagagcctc ttggttcaag tgacccttgg
4801  gctgctaatg aacctcggtg cctcttgtcc ccatctgtaa acaggggaaa
4851  taatagtgct gtgtcctaag ggttattgtt tggatcagtg aggtaactca
4901  agttgaatgc ttagaacagc ccatcatacg tacatggtac ccaataaatg
4951  ctagccactg tgttatgact gccccacctc tgcaccccaa gttcctgagc
5001  ctccccttca ctccactttg acacggcccc tcccttgtga cctgagggca
                                EXON 12
5051  ggtccccact ctgtcctggc agGAGCGCAT GGGCGAGTGT CTGCAGCGCA
```

Fig. 1 (cont.)

```
5101  CGGGCACCAG TGTCGTACTC ACATCCATCA ACAACATGGC CGCCTTCCTC
5151  ATGGCTGCCC TCGTTCCCAT CCCTGCGCTG CGAGCCTTCT CCCTACAGGC
5201  GGCCATAGTG GTTGGCTGCA CCTTTGTAGC CGTGATGCTT GTCTTCCCAG
5251  CCATCCTCAG CCTGGACCTA CGGCGGCGCC ACTGCCAGCG CCTTGATGTG
5301  CTCTGCTGCT TCTCCAGgta ctgcgtgcgc cccagccccc tcctcccgtg
5351  acccacgcca gcctgtcccc tcaccagcat ttcaaggcac agacctgtca
                                EXON 13
5401  tccactctct acctcttcca gTCCCTGCTC TGCTCAGGTG ATTCAGATCC
5451  TGCCCCAGGA GCTGGGGGAC GGGACAGTAC CAGTGGGCAT TGCCCACCTC
5501  ACTGCCACAG TTCAAGCCTT TACCCACTGT GAAGCCAGCA GCCAGCATGT
5551  GGTCACCATC CTGCCTCCCC AAGCCCACCT GGTGCCCCCA CCTTCTGACC
5601  CACTGGGCTC TGAGCTCTTC AGCCCTGGAG GGTCCACACG GGACCTTCTA
5651  GGCCAGGAGG AGGAGACAAG GCAGAAGGCA GCCTGCAAGT CCCTGCCCTG
5701  TGCCCGCTGG AATCTTGCCC ATTTCGCCCG CTATCAGTTT GCCCCGTTGC
5751  TGCTCCAGTC ACATGCTAAG gtaagactgg gcagagcagg gcagagactt
5801  agcatctctg ggcccagaag ggcagagagg gcttagtcca ctgcctgagg
                                                        EX
5851  ggctgggggc agccctgggg tctccagctt agttgctaca tcccgcagGC
      XON 14
5901  CATCGTGCTG GTGCTCTTTG GTGCTCTTCT GGGCCTGAGC CTCTACGGAG
5951  CCACCTTGGT GCAAGACGGC CTGGCCCTGA CGGATGTGGT GCCTCGGGGC
6001  ACCAAGGAGC ATGCCTTCCT GAGCGCCCAG CTCAGGTACT TCTCCCTGTA
6051  CGAGGTGGCC CTGGTGACCC AGGGTGGCTT TGACTACGCC CACTCCCAAC
6101  GCGCCCTCTT TGATCTGCAC CAGCGCTTCA GTTCCCTCAA GGCGGTGCTG
6151  CCCCCACCGG CCACCCAGGC ACCCCGCACC TGGCTGCACT ATTACCGCAA
6201  CTGGCTACAG Ggtgagaggc gaggagacgg gcagggaggg gtgctgcagg
6251  gagaaacgcc ctggggccac cagctaatag aaccctatcc tggtctcccc
        EXON 15
6301  cagGAATCCA GGCTGCCTTT GACCAGGACT GGGCTTCTGG GCGCATCACC
6351  CGCCACTCGA CCGCAATGGC TCTGAGGATG GGGCCCTGGC CTACAAGCTG
6401  CTCATCCAGA CTGGAGACGC CCAGGAGCTT CTGGATTTCA GCCAGgttgg
```

Fig. 1 (cont.)

```
6451  gagagggctg gaggggtcca ctagtacagg ggctgcaggc ctcctgggcc
                                        EXON 16
6501  caggccttca gccctctctg cctctgcagC TGACCACAAG GAAGCTGGTG
6551  GACAGAGAGG GACTGATTCC ACCCGAGCTC TTCTACATGG GGCTGACCGT
6601  GTGGGTGAGC AGTGACCCCC TGGGTCTGGC AGCCTCACAG GCCAACTTCT
6651  ACCCCCCACC TCCTGAATGG CTGCACGACA AATACGACAC CACGGGGGAG
6701  AACTTTCGCA gtgagtcttg gggggagctc ggcaagagcc tcagcctcgc
6751  ccacacaagc cctgagcctg aggccctgcc cactctgccc cgtgctcacc
                                                  EXON 17
6801  gccctgtccc tctccctctt ctcccttccc ctcccctcca cagTCCCGCC
6851  AGCTCAGCCC TTGGAGTTTG CCCAGTTCCC TTTCCTGCTG CGTGGCCTCC
6901  AGAAGACTGC AGACTTTGTG GAGGCCATCG AGGGGGCCCG GGCAGCATGC
6951  GCAGAGGCCG GCCAGGCTGG GGTGCACGCC TACCCCAGCG GCTCCCCCTT
7001  CCTCTTCTGG GAACAGTATC TGGGCCTGCG GCGCTGCTTC CTGCTGGCCG
7051  TCTGCATCCT GCTGGTGTGC ACTTTCCTCG TCTGTGCTCT GCTGCTCCTC
7101  AACCCCTGGA CGGCTGGCCT CATAgtgagt gcttgcagga gtggggacag
7151  agacacccca cccttccctg cccagcctgt catccctcct gccaggagcc
                                  EXON 18
7201  ctctgtgagc cctgtctccc tcagGTGCTG GTCCTGGCGA TGATGACAGT
7251  GGAACTCTTT GGTATCATGG GTTTCCTGGG CATCAAGCTG AGTGCCATCC
7301  CCGTGGTGAT CCTTGTGGCC TCTGTAGGCA TTGGCGTTGA GTTCACAGTC
7351  CACGTGGCTC TGGGCTTCCT GACCACCCAG GGCAGCCGGA ACCTGCGGGC
7401  CGCCCATGCC CTTGAGCACA CATTTGCCCC CGTGACCGAT GGGGCCATCT
7451  CCACATTGCT GGGTCTGCTC ATGCTTGCTG GTTCCCACTT TGACTTCATT
7501  GTAAG..... ..........  ..........  ..........  ..........
7551  ..........  gtagggaggg ctcggggcag ggaggcaggg ctcaggacag
                                              EXON 20
7601  gcctgggctg actccccccа cacccтaccc ctagGTACTT CTTTGCGGCG
7651  CTGACAGTGC TCACGCTCCT GGGCCTCCTC CATGGACTCG TGCTGCTGCC
7701  TGTGCTGCTG TCCATCCTGG GCCCGCCGCC AGAGgtgacc acacctcgg
7751  caccatccct ctactcccag cccaagggac ggggtaggga gaggcaaggg
```

Fig. 1 (cont.)

```
7801  aagggacaga gccctgtggc ccacagacag gtacctcccc aacaggtgcc
7851  accagctgaa ggtggcagcc tcctcctttc cccagacacc atgttcctgc
7901  ccctcagccc tcctggcttc ttcatgggac ccaccttaga cttttaggat
7951  ccagaacaag gtgcagggtt tgccccaggc ctcaacatcc tgtcgcctgc
8001  cagctctcat atcctgctgg agaccaacaa gggccccagc ttcccaacag
8051  tcatggtaat ccccagcgag atgctaaagg ggacgggagc cccagggccc
                                    EXON 21
8101  cgtgggctta ctggggctgg tgtctccccа cagGTGATAC AGATGTACAA
8151  GGAAAGCCCA GAGATCCTGA GTCCACCAGC TCCACAGGGA GGCGGGCTTA
8201  Ggtggggggc atcctcctcc ctgccccaga gctttgccag agtgactacc
8251  tccatgaccg tggccatcca cccaccccсc ctgcctggtg cctacatcca
8301  tccagccccc gatgagcccc cttggtcccc tgctgtcact agctctggca
8351  acctcagttc caggggacca ggtccagcca ctgggtgaaa gagcagctga
8401  agcacagaga ccatgtgtgg ggcgtgtggg gtcactggga agcactgggt
8451  ctggtgttag acgcaggatg gacccctgga gggctctgct gctgctgcat
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8551  agccaccgat ttgcacatcc aggcctgtgt gagcctgtat ctgtgtcact
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8851  ttctatgtga agctatgatg aaagttttat tttttaaaga atgaaatata
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8951  ttctatgtgt gcaagtgaac attagcttca gttgcttttt tttggacaga
9001  gtggggagtt tgcaagtgaa cattagctat tggaaggagc ttctctggtg
9051  ccaggacctg aggtattagc ttctctagtt ctgggtggaa aagaccccag
9101  attctggatt tttgtcatat acttggtaac atcatctgga ttaagtgctt
```

Fig. 1 (cont.)

```
9151  actatacaaa acgataacaa attttgttgg tgtgaaatcc tactggttc
9201  aatctggaga ccgagagcag aaaaaaaaga accccactgt gtggctttca
9251  gagccaccat attccagcct gcccgtctct ccagactcac ctccacctac
9301  ctgcttcacc cgcacgggaa acggcaaggc agaggggcaa agccatgcag
9351  caggtggaag gcgaggtgga ggcagatcag gaaagcagcc agttgaagca
9401  gagagaggtc aacagggtct ggggagcttc tcaggaggtt tgtggaccca
9451  gggaaaggag ccaggttcca gagcaacctc caaggcaaag gcctctgtaa
9501  gttggttgtc ctgacagccg agaggtgtct ttggccagtc agccagtgga
9551  tcagttgcgg gaactgctca gaaactgagg tgctagcagt tagtgaggac
9601  acagcgtaag ttgtttgttc tgtgaaagtt gaacagctcc actaagcaga
9651  ggccttgaag agtggccaca gccctggaat agagcacaga gcctcaccta
9701  gaggcgtggg gaggtttgca actgcccctt cccagccata gcttaggacc
9751  catagtctag ttcacataga ccctgggctc caaccaccca ctcaccagga
9801  atgatcccac cccaggaaca atgcgttctc acatcccacc ccacctggac
9851  aaaggccagg aaatcatgtt ctgaccaaaa gatacaacaa caaaaacaac
9901  aacaacaaaa aacgcctatt gcaattgaat ccacgctaaa atgcctaaaa
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10001 ccagcaggcc caagggaggg aggctgcatt tgggtccagc agtgtttggg
10051 tcaccaagaa gggccttcta ggtggagcag agagagctca ccaggccaga
10101 atagtgcaaa gggggtcagc cctcagtgcc acttaccagc ggagtaaccc
                                                        E
10151 tgggcaagtt agccagcctc actaagcctc cccatcttca tctttccagG
      XON 22
10201 CCCGAGGAGA TCTAGCCTCT GCCTCCCACC CCAGCACCCC CTCATCAGAC
10251 ACAAGGAGCG CCACTGTCTG GACAGGCTGA ATTGGTCTTC GGGTCCCTAA
10301 TTTCTCATAC GCCATTCCCT CTGCCTAGAA CACTTTCTCA CCTCCCCTTG
10351 ATGTGACCCC ATATCACCCT TCGAGGTGAA TTGGATCGGA TGCCATCTCC
10401 TCCAGGAGGG GTGGGGTCGT GCCTCCTGTG AGGTCCCAGT GCCCCTGAGT
10451 GTCTGTGCCC GTCTGTTTCC CCGTCCCTCT CTCTAAGCCC GGAGGCTTAC
```

Fig. 1 (cont.)

```
10501  TGCGGGTAAG GACGGCGGGA CAGGACCTTA ACCCCTGGGA CGAACACCAG
10551  CTCCGCAAAG GACTCCGCAC CCGGCGCCGC CCACGGGGTG CGGGTCCCAG
10601  GAGGACCAGC AGAGAGGAGC ATAGGAGAGC AAAGGAGATC AGTGACCCAT
10651  GGCTTCCCCG GTGGCGCGGA ACAGCCCGGA GCCGCCTGTG ATTTGCATAC
10701  CCATGGTGCA CCACGAAAAG ATACCCTCAA GATGCTTGCA CTCCCTCTGT
10751  GCGCGCATTT CTGCACTGTT TTAGAGCATG ATGCCTCTTA CACGCATCTG
10801  TGTGCATAAA CTACATATAG GGAGTGCGTA CCACGCAGGC ATCCAACAAC
10851  CATAAGTGTG TTAAGTGTTA GTTCTCCCTG CGAGGTTCGA AGCGGAAGTC
10901  ACGAATATAC TCGGGTTTCT CTTCAAAGCG CATAAATCTT TCGCCTTTTA
10951  CTAAAGATTT CCGTGGAGAG AAAGTTGTGA GTTTTTATTC AATTTTTTGA
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11051  TACAAAAAAA AAAAAAAA
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Fig. 1 (cont.)

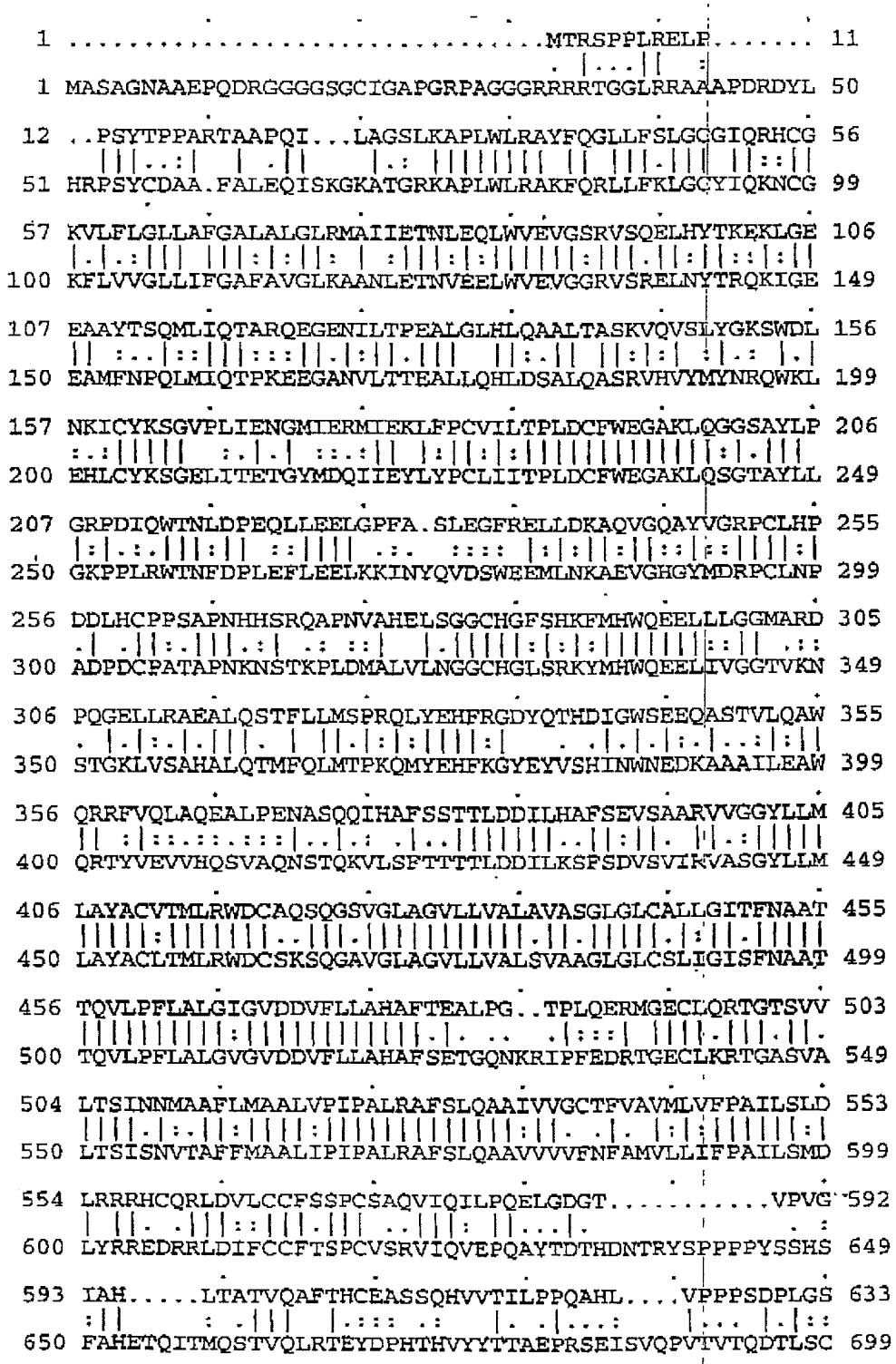


Fig. 2A

Fig. 2B Genomic

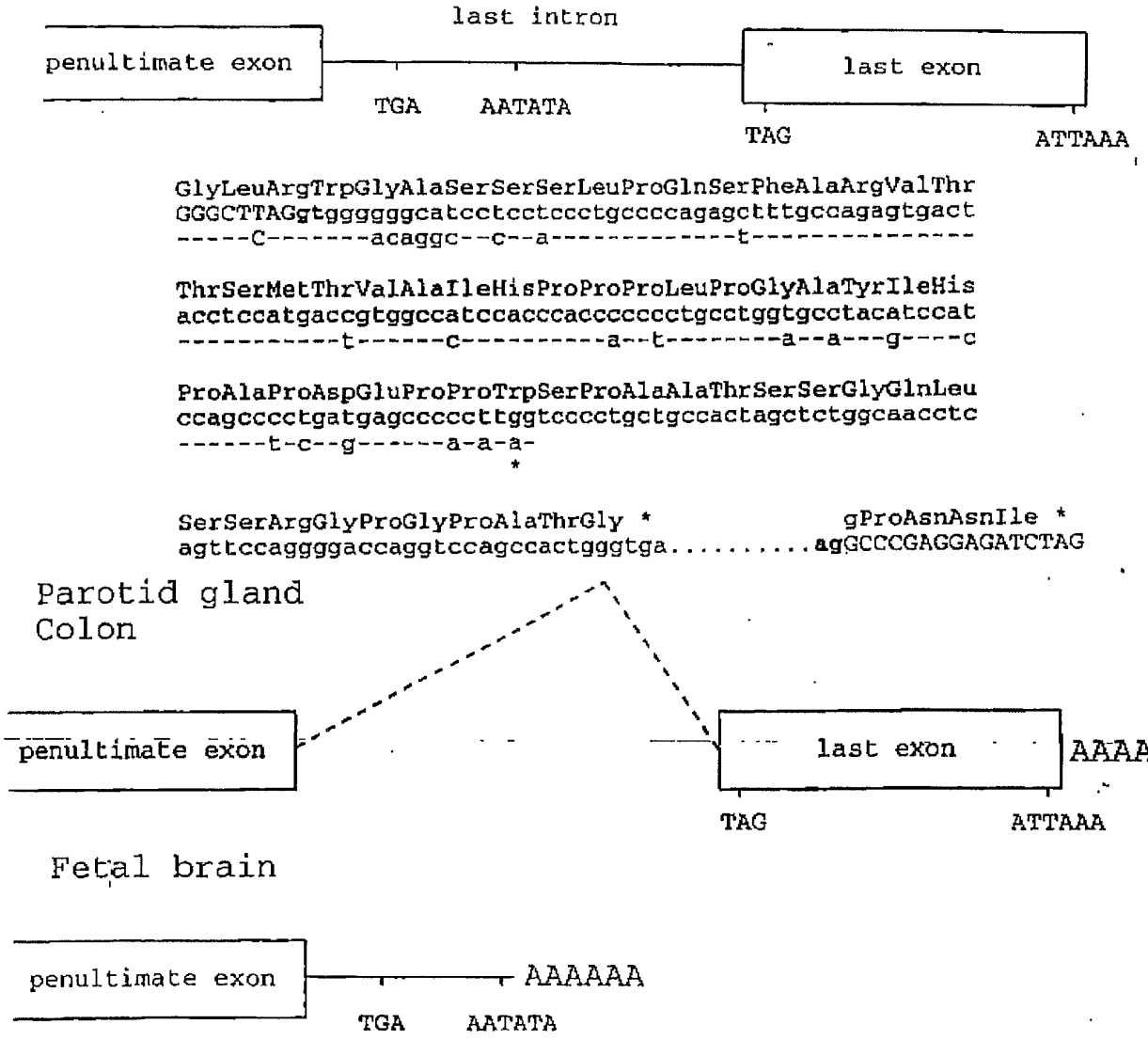
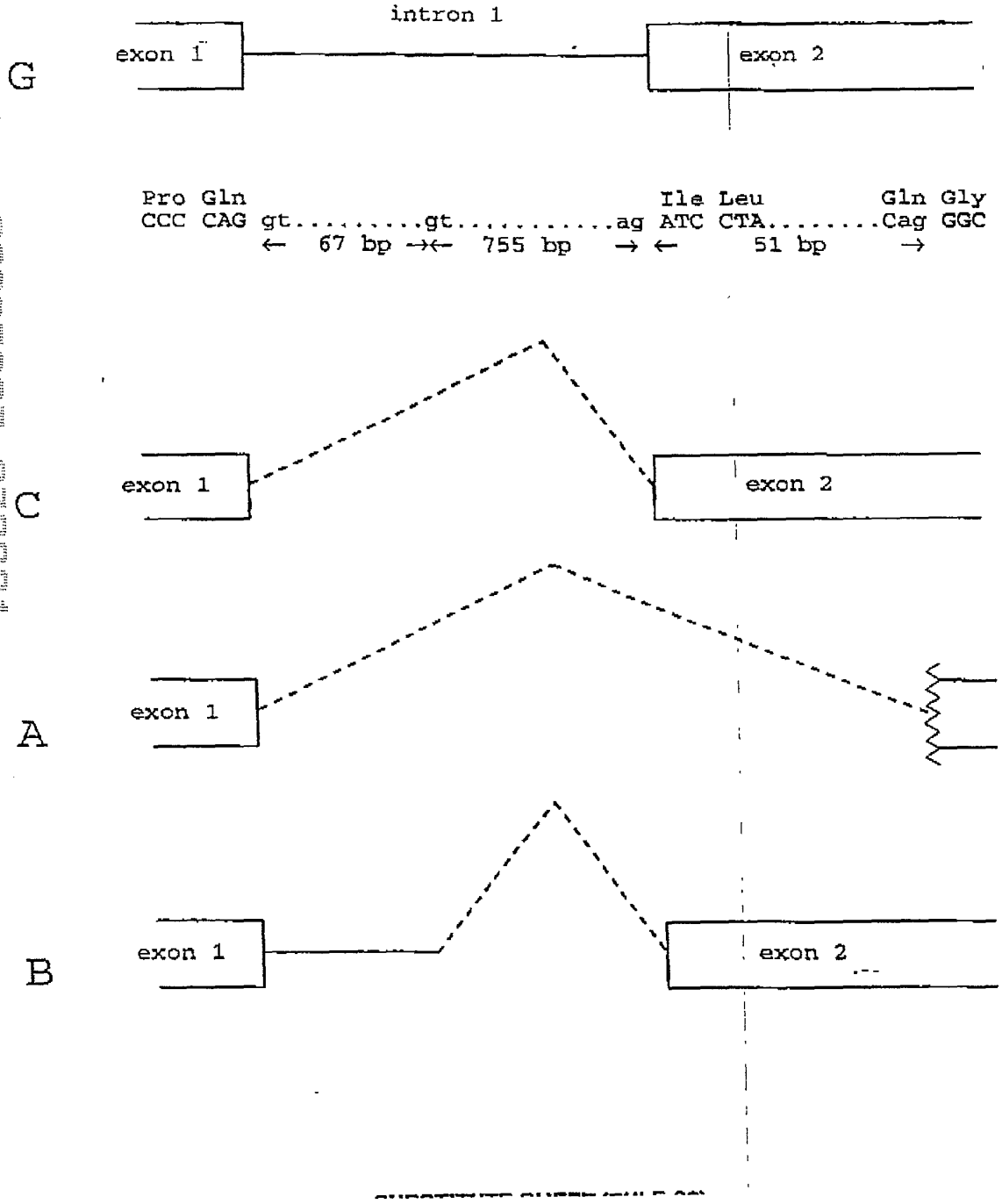


Fig. 2C


G
intron 1
exon 1
exon 2
Pro Gln
CCC CAG gt.....gt.....ag
Ile Leu
ATC CTA.....
Gln Gly
Cag GGC
← 67 bp →← 755 bp → ← 51 bp →
C
exon 1
exon 2
A
exon 1
B
exon 1
exon 2

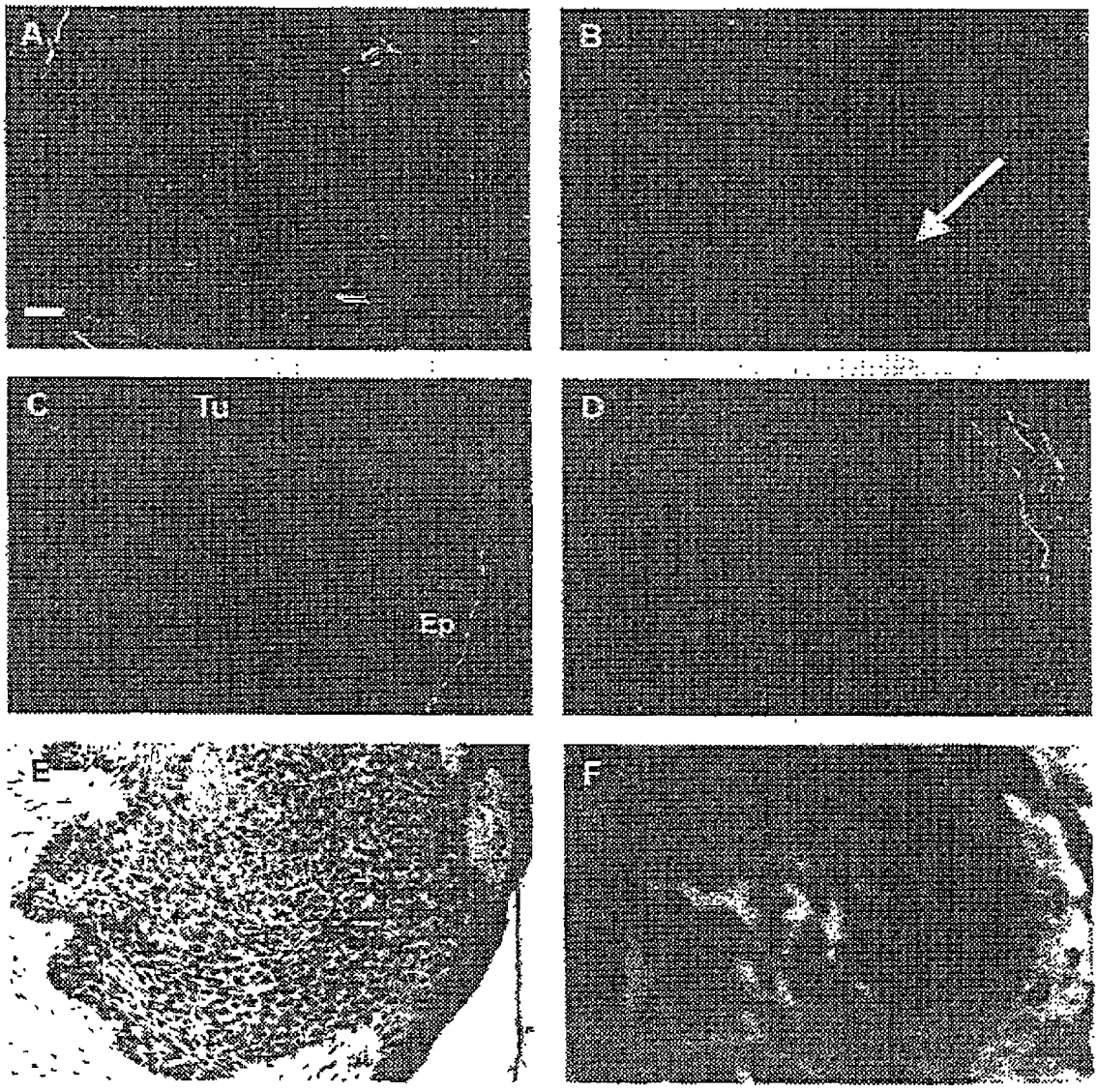


Fig. 3

Attorney Docket No.

BIRCH, STEWART, KOLASCH & BIRCH, LLP

P.O. Box 747 • Falls Church, Virginia 22040-0747
Telephone: (703) 205-8000 • Facsimile: (703) 205-8050

2921-0130P

PLEASE NOTE: YOU MUST COMPLETE THE FOLLOWING

COMBINED DECLARATION AND POWER OF ATTORNEY FOR PATENT AND DESIGN APPLICATIONS

As a below named inventor, I hereby declare that: my residence, post office address and citizenship are as stated next to my name; that I verily believe that I am the original, first and sole inventor (if only one inventor is named below) or an original, first and joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Insert Title: A NOVEL COMPONENT

Fill in Appropriate Information - For Use Without Specification Attached:

the specification of which is attached hereto. If not attached hereto,
the specification was filed on April 6, 2001 as
United States Application Number _____;
and amended on April 6, 2001 (if applicable) and/or
the specification was filed on October 6, 1999 as PCT
International Application Number PCT/SE99/01784; and was
amended under PCT Article 19 on _____ (if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I do not know and do not believe the same was ever known or used in the United States of America before my or our invention thereof, or patented or described in any printed publication in any country before my or our invention thereof or more than one year prior to this application, that the same was not in public use or on sale in the United States of America more than one year prior to this application, that the invention has not been patented or made the subject of an inventor's certificate issued before the date of this application in any country foreign to the United States of America on an application filed by me or my legal representative or assigns more than twelve months (six months for designs) prior to this application, and that no application for patent or inventor's certificate on this invention has been filed in any country foreign to the United States of America prior to this application by me or my legal representatives or assigns, except as follows.

I hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Insert Priority Information: (if appropriate)

Prior Foreign Application(s)			Priority Claimed	
9803393-0 (Number)	Sweden (Country)	October 6, 1998 (Month/Day/Year Filed)	☒ Yes	☐ No
_____ (Number)	_____ (Country)	_____ (Month/Day/Year Filed)	☐ Yes	☐ No
_____ (Number)	_____ (Country)	_____ (Month/Day/Year Filed)	☐ Yes	☐ No
_____ (Number)	_____ (Country)	_____ (Month/Day/Year Filed)	☐ Yes	☐ No

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional applications(s) listed below.

Insert Provisional Application(s): (if any)

_____ (Application Number) _____ (Filing Date)

_____ (Application Number) _____ (Filing Date)

All Foreign Applications, if any, for any Patent or Inventor's Certificate Filed More than 12 Months (6 Months for Designs) Prior to the Filing Date of This Application:

Insert Requested Information: (if appropriate)

Country	Application Number	Date of Filing (Month/Day/Year)

I hereby claim the benefit under Title 35, United States Code, §120 of any United States and/or PCT application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States and/or PCT application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose information which is material to the patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

Insert Prior U.S. Application(s): (if any)

(Application Number)	(Filing Date)	(Status - patented, pending, abandoned)

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I hereby appoint the following attorneys to prosecute this application and/or an international application based on this application and to transact all business in the Patent and Trademark Office connected therewith and in connection with the resulting patent based on instructions received from the entity who first sent the application papers to the attorneys identified below, unless the inventor(s) or assignee provides said attorneys with a written notice to the contrary:

Raymond C. Stewart	(Reg. No. 21,066)	Terrell C. Birch	(Reg. No. 19,382)
Joseph A. Kolasch	(Reg. No. 22,463)	James M. Slattery	(Reg. No. 28,380)
Bernard L. Sweeney	(Reg. No. 24,448)	Michael K. Mutter	(Reg. No. 29,680)
Charles Gorenstein	(Reg. No. 29,271)	Gerald M. Murphy, Jr.	(Reg. No. 28,977)
Leonard R. Svensson	(Reg. No. 30,330)	Terry L. Clark	(Reg. No. 32,644)
Andrew D. Meikle	(Reg. No. 32,868)	Marc S. Weiner	(Reg. No. 32,181)
Joe McKinney Muncy	(Reg. No. 32,334)	Donald J. Daley	(Reg. No. 34,313)
John W. Bailey	(Reg. No. 32,881)	John A. Castellano	(Reg. No. 35,094)
Gary D. Yacura	(Reg. No. 35,416)		

Send Correspondence to:

BIRCH, STEWART, KOLASCH & BIRCH, LLP or **Customer No. 2292**
P.O. Box 747 • Falls Church, Virginia 22040-0747
Telephone: (703) 205-8000 • Facsimile: (703) 205-8050

PLEASE NOTE: YOU MUST COMPLETE THE FOLLOWING:

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full Name of First or Sole Inventor: Insert Name of Inventor → Insert Date This Document is Signed

GIVEN NAME/FAMILY NAME	INVENTOR'S SIGNATURE	DATE*
Peter G. ZAPHIROPOULOS		2001-05-10
Residence (City, State & Country)		CITIZENSHIP
Tullinge, Sweden SE		Greek
MAILING ADDRESS (Complete Street Address including City, State & Country)		
Tullinge Strand 96, S-146 54 Tullinge, SWEDEN		

Insert Residence Insert Citizenship →

Insert Post Office Address →

Full Name of Second Inventor, if any: see above

GIVEN NAME/FAMILY NAME	INVENTOR'S SIGNATURE	DATE*
Anne Birgitte UNDEN	Anne Birgitte Unden	2001-05-10
Residence (City, State & Country)		CITIZENSHIP
Djursholm, Sweden SE		Norwegian
MAILING ADDRESS (Complete Street Address including City, State & Country)		
Gandviksvägen 3, S-181 62 Djursholm, SWEDEN		

Full Name of Third Inventor, if any: see above

GIVEN NAME/FAMILY NAME	INVENTOR'S SIGNATURE	DATE*
Rune TOFTGÅRD		2001-05-10
Residence (City, State & Country)		CITIZENSHIP
Skärholmen, Sweden SE		Swedish
MAILING ADDRESS (Complete Street Address including City, State & Country)		
Sätragårdsvägen 209, S-127 36 Skärholmen, SWEDEN		

Full Name of Fourth Inventor, if any: see above

GIVEN NAME/FAMILY NAME	INVENTOR'S SIGNATURE	DATE*
Fahimeh, RAHNAMA	F. Rahnama	2001-05-10
Residence (City, State & Country)		CITIZENSHIP
Stockholm, Sweden SE		Swedish
MAILING ADDRESS (Complete Street Address including City, State & Country)		
Stadshagvägen 18 3 tr, S-112 50 Stockholm, SWEDEN		

*DATE OF SIGNATURE

Attorney Docket No.

5 of 10

Full Name of Fifth Inventor, if any see above

GIVEN NAME/FAMILY NAME	INVENTOR'S SIGNATURE	DATE*
Robert E. HOLLINGSWORTH	Robert E. Hollingsworth	5-22-01
~~Residence~~ (City, State & Country)		CITIZENSHIP
NC 27709, USA		US
MAILING ADDRESS (Complete Street Address including City, State & Country)		
Glaxo Inc. 5 Moore Drive, Research Triangle Park, USA NC27709		

Full Name of Sixth Inventor, if any see above

GIVEN NAME/FAMILY NAME	INVENTOR'S SIGNATURE	DATE*
Residence (City, State & Country)		CITIZENSHIP
MAILING ADDRESS (Complete Street Address including City, State & Country)		

Full Name of Seventh Inventor, if any see above

GIVEN NAME/FAMILY NAME	INVENTOR'S SIGNATURE	DATE*
Residence (City, State & Country)		CITIZENSHIP
MAILING ADDRESS (Complete Street Address including City, State & Country)		

Full Name of Eighth Inventor, if any see above

GIVEN NAME/FAMILY NAME	INVENTOR'S SIGNATURE	DATE*
Residence (City, State & Country)		CITIZENSHIP
MAILING ADDRESS (Complete Street Address including City, State & Country)		

Full Name of Ninth Inventor, if any see above

GIVEN NAME/FAMILY NAME	INVENTOR'S SIGNATURE	DATE*
Residence (City, State & Country)		CITIZENSHIP
MAILING ADDRESS (Complete Street Address including City, State & Country)		

Full Name of Tenth Inventor, if any see above

GIVEN NAME/FAMILY NAME	INVENTOR'S SIGNATURE	DATE*
Residence (City, State & Country)		CITIZENSHIP
MAILING ADDRESS (Complete Street Address including City, State & Country)		

Full Name of Eleventh Inventor, if any see above

GIVEN NAME/FAMILY NAME	INVENTOR'S SIGNATURE	DATE*
Residence (City, State & Country)		CITIZENSHIP
MAILING ADDRESS (Complete Street Address including City, State & Country)		

Full Name of Twelfth Inventor, if any see above

GIVEN NAME/FAMILY NAME	INVENTOR'S SIGNATURE	DATE*
Residence (City, State & Country)		CITIZENSHIP
MAILING ADDRESS (Complete Street Address including City, State & Country)		

*DATE OF SIGNATURE